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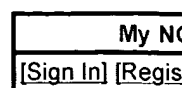
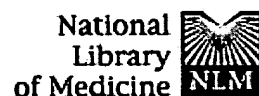
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| L27 | 1 | minimum WITH I3             | US-PGPUB;<br>USPAT;<br>EPO; JPO;<br>DERWENT | OR | ON | 2005/05/24 15:19 |

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| 2  | US<br>20040048261<br>A1 | Invertebrate choline transporter nucleic acids, polypeptides and uses thereof                                      |
| 3  | US<br>20030217376<br>A1 | Insecticide targets and methods of use   |
| 4  | US<br>20020173634<br>A1 | Methods and compositions for transposition using minimal segments of the eukaryotic transformation vector piggybac |
| 5  | US<br>20020009751<br>A1 | Drosophila homologues of genes and proteins implicated in metabolism and methods of use                            |
| 6  | US 6762291 B1           | Insect p53 tumor suppressor genes and proteins   |
| 7  | US 6599717 B1           | Invertebrate vascular endothelial growth factor receptor   |
| 8  | US 6579701 B1           | Drosophila homologues of genes and proteins implicated in cancer and methods of use                                |
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| 10 | US 6518064 B1           | Pink bollworm expression system for commercially valuable protein production                                       |

|           | Document ID   | Title   |
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| <b>12</b> | US 6218185 B1 | Piggybac transposon-based genetic transformation system for insects             |



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1: Insect Mol Biol. 1999 Nov;8(4):449-57.

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## Germline transformation of *Drosophila melanogaster* with the piggyBac transposon vector.

Handler AM, Harrell RA 2nd.

Center for Medical, Agricultural, and Veterinary Entomology, US  
Department of Agriculture, Gainesville, FL 32608, USA.  
handler@nersp.nerdc.ufl.edu

Germline transformation of *Drosophila melanogaster* was attempted with the piggyBac gene-transfer system from the cabbage looper moth, *Trichoplusia ni*. Using a self-regulated transposase helper and a white marked vector, a transformation frequency of 1-3% per fertile G0 was obtained, similar to that previously achieved in the medfly. Use of an hsp70-regulated helper increased this frequency more than eight-fold. Transformation with a vector marked with white and green fluorescent protein (GFP) under polyubiquitin-nuclear localizing sequence regulation yielded seventy G1 transformants which all expressed GFP, but only twenty-seven of these expressed eye pigmentation that would have allowed their selection based on white+ expression. PiggyBac transformation in two distantly related dipteran species and efficient expression of the gfp marker supports the potential use of this system in other dipterans, and perhaps insects in general.

PMID: 10634970 [PubMed - indexed for MEDLINE]

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transformation of an insect by the **piggyBac transposon** was accomplished ...

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for transformation constructs containing **piggyBac** transposable elements. ...

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(19) **United States**

(12) **Patent Application Publication**

**Fraser, JR. et al.**

(10) **Pub. No.: US 2002/0173634 A1**

(43) **Pub. Date: Nov. 21, 2002**

(54) **METHODS AND COMPOSITIONS FOR  
TRANSPPOSITION USING MINIMAL  
SEGMENTS OF THE EUKARYOTIC  
TRANSFORMATION VECTOR PIGGYBAC**

(76) **Inventors: Malcolm J. Fraser JR., Granger, IN  
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Teresa Beam, Columbia City, IN (US);  
Aurelie Hua-Van, Cedex (FR)**

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(21) **Appl. No.: 10/001,189**

(22) **Filed: Oct. 30, 2001**

**Related U.S. Application Data**

(60) **Provisional application No. 60/244,984, filed on Nov.  
1, 2000. Provisional application No. 60/244,677, filed  
on Oct. 31, 2000.**

**Publication Classification**

(51) **Int. Cl.<sup>7</sup> ..... C07H 21/02; C07H 21/04**  
(52) **U.S. Cl. .... 536/23.1**

(57) **ABSTRACT**

More efficient transfer of genes into host cells or embryos to transform the cells or embryos is facilitated by transposition vectors using the minimal amount of nucleotide sequences in the transposon piggyBac required for gene transfer. The transformed cells or embryos may be developed into transgenic organisms.

FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 15:22:04 ON 24 MAY 2005

L1 34541 S HANDLER?/AU OR BEAM?/AU OR HUA-VAN?/AU OR LI-XU?/AU OR FRASER  
L2 173 S PIGGYBAC  
L3 25372 S TRANSPOSON  
L4 5 S (MINIMUM OR INTERNAL) (S) L2  
L5 2 DUP REM L4 (3 DUPLICATES REMOVED)  
L6 51 S L1 AND L2  
L7 5 S L6 AND ((MINIMUM OR INTERNAL (S) PIGGYBAC))  
L8 2 DUP REM L7 (3 DUPLICATES REMOVED)  
L9 674004 S FLUORESCENT OR FLUORESCENCE  
L10 79355 S HEAT (2W) SHOCK  
L11 292 S UBIQUITIN (2W) PROMOTER  
L12 157408 S DROSOPHILA  
L13 292 S L11 (P) L11  
L14 108 S BGLII (S) HPAI  
L15 0 S L14 AND L2  
L16 0 S L14 AND L1  
L17 5 S L14 AND L3  
L18 3 DUP REM L17 (2 DUPLICATES REMOVED)  
L19 0 S L9 AND L10 AND L11 AND L12  
L20 0 S L9 AND L10 AND L11  
L21 4584 S L12 AND L9  
L22 70 S L21 AND L3  
L23 0 S L22 AND L11  
L24 77 S L2 AND L12  
L25 16 S L24 NOT PY>=2001  
L26 9 DUP REM L25 (7 DUPLICATES REMOVED)  
L27 0 S L26 AND (DELET? (5W) SEQUENCE)

=>

L5 ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1  
 ACCESSION NUMBER: 2005037456 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 15663772  
 TITLE: **piggyBac internal** sequences are necessary for efficient transformation of target genomes.  
 AUTHOR: Li X; Harrell R A; Handler A M; Beam T; Hennessy K; Fraser M J Jr  
 CORPORATE SOURCE: Department of Biological Sciences, and Center for Tropical Diseases Research and Training, University of Notre Dame, Notre Dame, IN 46556, USA.  
 CONTRACT NUMBER: AI48561 (NIAID)  
 SOURCE: Insect molecular biology, (2005 Jan) 14 (1) 17-30. Journal code: 9303579. ISSN: 0962-1075.  
 PUB. COUNTRY: England: United Kingdom  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200503  
 ENTRY DATE: Entered STN: 20050125  
 Last Updated on STN: 20050325  
 Entered Medline: 20050324

AB A previously reported **piggyBac** minimal sequence cartridge, which is capable of efficient transposition in embryo interplasmid transposition assays, failed to produce transformants at a significant frequency in *Drosophila melanogaster* compared with full-length or less extensive **internal** deletion constructs. We have re-examined the importance of these internal domain (ID) sequences for germline transformation using a PCR strategy that effectively adds increasing lengths of ID sequences to each terminus. A series of these piggyBac ID synthetic deletion plasmids containing the 3xP3-ECFP marker gene are compared for germline transformation of *D. melanogaster*. Our analyses identify a minimal sequence configuration that is sufficient for movement of piggyBac vectored sequences from plasmids into the insect genome. Southern hybridizations confirm the presence of the piggyBac transposon sequences, and insertion site analyses confirm these integrations target TTAA sites. The results verify that ID sequences adjacent to the 5' and 3' terminal repeat domains are crucial for effective germline transformation with piggyBac even though they are not required for excision or interplasmid transposition. Using this information we reconstructed an inverted repeat cartridge, ITR1.1k, and a minimal piggyBac transposon vector, pXL-BacII-ECFP, each of which contains these identified ID sequences in addition to the terminal repeat configuration previously described as essential for mobility. We confirm in independent experiments that these new minimal constructs yield transformation frequencies similar to the control piggyBac vector. Sequencing analyses of our constructs verify the position and the source of a point mutation within the 3' internal repeat sequence of our vectors that has no apparent effect on transformation efficiency.

L5 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 2  
 ACCESSION NUMBER: 2001609493 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 11683259  
 TITLE: The **minimum internal** and external sequence requirements for transposition of the eukaryotic transformation vector **piggyBac**.  
 AUTHOR: Li X; Lobo N; Bauser C A; Fraser M J Jr  
 CORPORATE SOURCE: Department of Biological Sciences, and Center for Tropical Diseases Research and Training, University of Notre Dame, IN 46556, USA.  
 CONTRACT NUMBER: AI 40960-01 (NIAID)  
 SOURCE: Molecular genetics and genomics : MGG, (2001 Oct) 266 (2) 190-8. Journal code: 101093320. ISSN: 1617-4615.  
 PUB. COUNTRY: Germany: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200112

ENTRY DATE:           Entered STN: 20011102  
                      Last Updated on STN: 20020123  
                      Entered Medline: 20011204

AB   The piggyBac element from *Trichoplusia ni* is recognized as a useful vector for transgenesis of a wide variety of species. This transposable element is 2472 bp in length, and has a complex repeat configuration consisting of an internal repeat (IR), spacer, and terminal repeat (TR) at both ends, and a single ORF encoding the transposase. Excision assays performed in microinjected *T. ni* embryos using plasmids deleted for progressively larger portions of the **piggyBac internal** sequence reveal that the 5' and 3' IR, spacer, and TR configuration is sufficient for precise excision of **piggyBac** when transposase is provided in trans. Interplasmid transposition assays using plasmids carrying varying lengths of intervening sequence between the **piggyBac** termini in *T. ni* demonstrate that a **minimum** of 55 bp of intervening sequence is required for optimal transposition, while lengths less than 40 bp result in a dramatic decrease in transposition frequency. These results suggest that the piggyBac transposase may bind both termini simultaneously before cleavage can occur, and/or that the formation of a transposition complex requires DNA bending between the two termini. Based on these results we constructed a 702-bp cartridge with minimal piggyBac 5' and 3' terminal regions separated by an intervening sequence of optimal length. Interplasmid transposition assays demonstrate that the minimal terminal configuration is sufficient to mediate transposition, and also verify that simply inserting this cartridge into an existing plasmid converts that plasmid into a non-autonomous piggyBac transposon. We also constructed a minimal **piggyBac** vector, pXL-Bac, that contains an **internal** multiple cloning site sequence between the minimal terminal regions. These vectors should greatly facilitate the utilization of the piggyBac transposon in a wide range of hosts.

=>



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